

role in transcription of genes involved in cardiac hypertrophy. The goal of this work is to investigate the relative role of nuclear InsP₃ in hypertrophy response induced by endothelin-1 (ET-1). For that, we used an adenovirus construct that has the ability to act as selective InsP₃ buffer in the nucleus (InsP₃-sponge-NLS), inducing local changes in Ca²⁺ levels. We used a primary culture of neonatal cardiomyocytes, and cells were examined by confocal microscopy and immunofluorescence. We found that ET-1 increased cell surface area by $54.3 \pm 6.87\%$ compared to control ($p < 0.05$), and that InsP₃-sponge-NLS prevented hypertrophy induced by ET-1. We also found that ANP expression levels remained at control levels when ET-1 treated cardiomyocytes had InsP₃ buffered in the nucleus. Then, we investigated whether buffering nuclear InsP₃ would affect the signaling pathway as calcineurin (Cn)/ nuclear factor of activated T cells (NFAT) and histone deacetylase 5 (HDAC-5) preventing the activation of hypertrophic genes. We observed that buffering nuclear InsP₃ decreased translocation of Cn, and NFAT (1.22 ± 0.13 and 0.46 ± 0.16 pixel/nuclear area for control and buffered cells, respectively, $p < 0.001$) upon ET-1 stimulation. On the other hand, the HDAC-5 exportation to cytosol was prevented by InsP₃-sponge-NLS (2.965 ± 0.13 and 5.548 ± 0.25 pixel/nuclear area for ET-1 treated cells and InsP₃-sponge-NLS infected plus ET-1 cardiomyocytes, respectively, $p < 0.001$). Together, these results show that nuclear InsP₃ plays a central role in the hypertrophic effect induced by ET-1. Support: HHMI/CNPq/Fapemig/CAPEs.

2816-Plat

Ca²⁺ Binding and Transport: A Novel Function for Coenzyme Q
Ivan Bogeski¹, Rubin Gulaboski¹, Valentin Mirceski², Reinhard Kapp¹, Markus Hoth¹.

¹Saarland University, Homburg, Germany, ²Ss. Cyril and Methodius University, Skopje, Macedonia, Germany.

Coenzyme Q10 (CoQ10) is one of the essential components of the mitochondrial electron-transport chain (ETC) with a primary function to transfer electrons along and protons across the inner mitochondrial membrane (IMM). The concomitant proton gradient across the IMM is essential for the process of oxidative phosphorylation and consequently ATP production.

We report that CoQ10 and its analog CoQ1 have an as-of-yet unknown potential in Ca²⁺ binding and transport. Using voltammetry, UV-VIS spectrometry, electron paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR) we show that the native form of both CoQ10 and its analog CoQ1 neither bind nor transport Ca²⁺ ions. However, when exposed to alkaline media or monooxygenase enzymes such as cytochrome P450, both CoQs undergo structural changes through a complex reaction pathway and form quinone structures with distinct properties. In a time dependent manner, one or both methoxy groups at position 2 and 3 on the quinone ring are replaced by a hydroxyl group. Contrary to the native form, the new hydroxylated products (of CoQ1 or CoQ10), depending on its redox transformation, are effectively chelating and transporting Ca²⁺ across artificial biomimetic membranes.

Our results open new perspectives about the physiological importance of CoQ10, not only as electron and proton transporter, but also as a potential regulator of mitochondrial Ca²⁺ homeostasis.

2817-Plat

Calcium Signalling in Red Blood Cells

Lars Kaestner¹, Patrick Steffen², Jue Wang¹, Asya Makhro³, Achim Jung², Duc Bach Nguyen², Ingolf Bernhardt², Anna Bogdanova³, Peter Lipp¹, Christian Wagner².

¹Saarland University, Homburg/Saar, Germany, ²Saarland University, Saarbrücken, Germany, ³University of Zürich, Zürich, Switzerland.

Thrombus formation is believed to be the major cause of stroke and infarction. Current understanding imposes mechanisms based on leucocyte and platelet activity. However, red blood cells make up a significant part of the clot. An active participation of red blood cells was hypothesised.

Cell based methods, namely the patch-clamp technique and observing as well as manipulating optical methods (video-imaging and holographic laser tweezers, respectively) were used to characterise human red blood cells to explore cellular signalling and cellular interactions.

Extracellular signalling molecules such as lysophosphatidic acid, prostaglandin E₂ or homocysteine can activate cation channels in the red blood cell membrane. The consecutive calcium influx and accumulation shows a distinct cell to cell variation throughout the red cell population. The calcium signal results in several cellular responses: (i) cell shrinkage due to the Gardos-channel activity, (ii) lipid asymmetry breakdown and phosphatidyl serin exposure to the outer membrane leaflet due to scramblase activity and (iii) irreversible cellular adhesion within individual red blood cells. The adhesion mechanism can just be speculated about. Using atomic force spectroscopy we measured an adhesion force of approximately 100 pN.

We provide further evidence derived from single cell experiments for the active participation of red blood cells in thrombotic events. In light of an increasing number of clinical indications, especially under pathophysiological conditions, such as sickle cell disease, thalassemia or dialysis patients, red blood cells may slowly move into the focus of comprehensive pharmacological strategies for preventing such thrombosis related events such as stroke and cardiac infarction.

Platform AZ: Member-organized Session: Disordered and Self-aggregated Peptides and Proteins

2818-Plat

The Evolution of the Natively Disordered Region in P53 Family Proteins
Buyong Ma, Ruth Nussinov.

SAIC-Frederick, NCI-Frederick, NIH, Frederick, MD, USA.

Protein disorder in N- and C-terminal regions is among the major structural factors that coupled to the amino acid evolution in p53. Using three independent predictors of protein disorder (Foldunfold, IUPred, and Foldindex), we found that the percentage of disordered fragments in p53 steadily increases with evolution, which is not the case for p63/p73. The increase in natively disordered segments may help p53 interact with many partners while making it less sensitive to mutational perturbations.

2819-Plat

Conformational Discrepancies Between Molecular Dynamics Force Fields and Vibrational Spectroscopy in Short Alanine-Based Peptides

Daniel Verbaro¹, Indrajit Gosh², Werner Nau², Reinhard Schweitzer-Stenner¹.

¹Drexel University, Philadelphia, PA, USA, ²Jacobs University, Bremen, Germany.

Structural preferences in the unfolded state of peptides determined by molecular dynamics still contradict experimental data. A remedy in this regard has been suggested by MD simulations with an optimized Amber force field ff03* (R. Best and G. Hummer, J. Phys. Chem. B 113, 9004-9015). The simulations yielded a statistical coil distribution for alanine, which is at variance with recent experimental results. To check the validity of this distribution, we investigated the peptide H-A5W-OH, which with the exception of the additional terminal tryptophan is analogous to the peptide used to optimize the force field. Electronic circular dichroism, vibrational circular dichroism, infrared spectroscopy as well as J-coupling constants obtained from NMR experiments were used to derive the peptide's conformational ensemble. Qualitatively, the experimental 3J(HN,Cα), VCD, and ECD indicated a preference of alanine for polyproline II-like conformations. Additionally, Förster-resonance-energy transfer between the terminal fluorophores of another analogous peptide Dbo-A5W-OH was used to determine its average length. In order to check whether the above statistical coil distribution quantitatively accounts for experimental data, we employed an excitonic model to calculate the amide I' profiles of the IR and VCD spectrum of H-A5W-OH as well as the distance between the two terminal peptide carbonyls by using the distribution obtained from ff03*. This led to an underestimated negative VCD couplet and an overestimated distance between terminal carbonyl groups. A better representation of experimental data was desired so we changed the distribution parameters in line with results recently obtained for alanine in GAG. This distribution model satisfactorily reproduced the amide I' profiles, the J-coupling constant and the end-to-end distance of H-A5W-OH, which reinforces alanine's high structural preference for polyproline II.

2820-Plat

The Role of Dynamic Protein Complexes in the Ubiquitin-Proteasome Pathway

Tanja Mittag¹, Stephen Orlicky², Xiaojing Tang², Frank Sicheri², Mike Tyers³, Julie D. Forman-Kay⁴.

¹St. Jude Children's Research Hospital, Memphis, TN, USA, ²Samuel Lunenfeld Research Institute, Toronto, ON, Canada, ³Wellcome Trust Centre for Cell Biology, Edinburgh, United Kingdom, ⁴The Hospital for Sick Children, Toronto, ON, Canada.

Intrinsically disordered proteins (IDPs) have been implicated in the regulation of many important cellular processes, such as regulation of cell cycle progression, of transcription and of translation. IDPs are thought to function primarily in the mediation of protein-protein interactions, but detailed analysis of the molecular mechanisms that render disorder beneficial in protein interactions is required. According to a common view, IDPs are usually thought to undergo disorder-to-order transitions upon interaction with their binding partners. It is becoming increasingly clear that predominantly disordered protein complexes